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Surveying viruses on ornamental trees and shrubs in two Hungarian botanical gardens and an arboretum

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Abstract

In Hungary the most common disease of fruit trees causing the most severe damages is *Plum pox virus* (PPV). This is why it is important to know other woody host plants that can be considered as source of infection. National surveys have been carried out since 2002 for revealing the distribution of PPV on ornamental and wild *Prunus* species.

From 2005 this work has been extended to studying other viruses on other woody plants. In two botanical gardens and an arboretum we selected plants (species and varieties) showing virus symptoms among various thousands of woody plants and submitted them to indexing on woody and herbaceous indicators, as well as to testing with ELISA for the presence of the following 11 viruses occurring on woody plants: PPV, *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Cherry leafroll virus* (CLRV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Apple mosaic virus* (ApMV), *Apple chlorotic leafspot virus* (ACLSV), *Strawberry latent ringspot virus* (SLRV), *Tomato black ring virus* (TBRV) and *Arabis mosaic virus* (ArMV).

Up to now, in 28 plant species and varieties, PPV, PDV, PNRSV, CLRV and ASPV have been detected so far. The presence of PPV was found in 9 species/varieties, such as *Prunus cerasifera* 'Pendula', *P. cerasifera* 'Pissardii', *P. glandulosa*, *P. glandulosa* 'Alba Plena', *P. glandulosa* 'Sinensis', *P. japonica*, *P. sogdiana*, *P. tomentosa* (from Tibet) and *P. x blireana*.

Seventeen species/varieties were found to be infected with PDV: *Lonicera caucasica*, *L. maackii*, *L. sachalinensis*, *Prunus mume* 'Omoi-no-mama', *P. salicina* (from China), *P. spinosa* 'Plena', *P. spinosa* 'Purpurea', *P. serrulata* 'Amanogawa', *P. serrulata* 'Ichiyō', *P. serrulata* 'Pink Perfection', *P. serrulata* 'Taihaku', *P. serrulata* 'Yedo-sakura', *P. subhirtella* 'Plena', *P. tenella*, *P. yedoensis*, *P. yedoensis* 'Moerheimii' and *Syringa yunnanensis*. Certain species/varieties infected with PDV were positive also for CLRV, such as *P. spinosa* 'Purpurea', *P. yedoensis*, *P. yedoensis* 'Moerheimii', *P. subhirtella* 'Plena', *P. serrulata* 'Yedo-sakura' and to ASPV: *P. subhirtella* 'Plena' and *P. serrulata* 'Yedo-sakura'. PNRSV was detected in *P. cerasifera* 'Nigra' and 'Hollywood'.

The difference of symptoms provoked by PDV on herbaceous hosts suggests that various strains of this virus affect hosts, mainly yellow mottle disease described as a separate strain by Ramaswamy and Posnette on ornamental cherries in 1972. Molecular studies are in progress to confirm the above results.

Keywords: ornamental trees and shrubs, virus symptoms, ELISA, *Prunus*, *Lonicera*, *Syringa*

Introduction

Plum pox virus (PPV) is widespread on stone fruit species causing severe losses in Hungary (Németh, 1986). In the last decades the occurrence of the virus on some ornamental and wild *Prunus* species has also been recorded in Hungary (Salamon and Palkovics, 2002; Sebestyén et al., 2008) as well as in other countries (Labonne et al., 2004; James and Thompson, 2006; Damsteegt et al., 2007). The infection is often symptomless.

In 2002 a several-year-survey was started at the Hungarian Plant Protection and the Soil Conservation Service in order to determine the eventual PPV infection of ornamental *Prunus* species (Sebestyén et al., 2008). From 2005 this work has been extended to studying other viruses on other woody plants. At the same time this work aimed to compile an illustrated diagnostic manual to assist the service staff in inspecting ornamental trees and shrubs. In two botanical gardens and an arboretum, plants (species and varieties) showing virus symptoms among various thousands of woody plants were selected and submitted to indexing on woody and herbaceous indicators, as well as to testing with ELISA for the presence of the 11 viruses (see below) occurring on woody plants.

Materials and methods

Surveys and sampling in propagation sites, such as nuclear stocks, propagation blocks, nurseries, arboretums, public areas including parks and street grown trees, were carried out by plant pathologists and inspectors of the county services in at least one location of each county between 2002 and 2007. Several thousand woody plants were visually checked during 2005 and 2008. Examinations took place three times a year in May, August and early October. The species and varieties showing virus symptoms were submitted to further indexing on woody and herbaceous indicators, as well as to testing with ELISA.

Indexing took place in the testing nursery and the glasshouse of the Agricultural Office of County Fejér, Plant Protection and Soil Conservation Directorate. Virus transmission was performed with double budding and chip budding in the testing nursery from late July to early August. The woody indicators used in indexing were peach seedling GF 305, *Prunus avium* seedling, GF 31 myrobalan hybrid, *P. domestica* 'Italian prune' and *P. serrulata* 'Shirofugen'. In greenhouse indexing, one-year shoots were taken for mechanical transmissions in February. The herbaceous indicators applied were as follows: *Celosia argentea*, *Chenopodium foetidum*, *Ch. murale*, *Ch. quinoa*, *Cucumis sativus*, *Cyamopsis tetragonoloba*, *Momordica balsamina*, *Nicotiana benthamiana*, *N. clevelandii*, *N. langsdorffii*, *N. tabacum* 'Samsun', *N. tabacum* 'White Burley', *Petunia hybrida*, *Phaseolus vulgaris*, *Sesbania exaltata*, *Solanum symbriifolium*, *Tithonia speciosa*, *Torenia fournieri* and *Zinnia elegans*.

The leaf samples were tested in duplicates by DAS-ELISA using commercial kits (supplier: BIOREBA for PDV, PNRSV, CLRV, ASPV, ASGV, ApMV, ACLSV, SLRV, TBRV, ArMV and SEDIAG for TBRV) and by DASI-ELISA for PPV (supplier: DURVIZ-IVIA). Optical densities (OD) were recorded at 405 nm after 60 and 90 minutes on Labsystems Multiskan® PLUS reader. Samples with OD double that of the healthy control were considered ELISA-positive.

Tab. 1 Results of biological indexing and ELISA for the ornamental/wild tree and shrub species and cultivars with symptoms collected in two botanical gardens and an arboretum of the Hungarian Academy of Sciences between 2002 and 2008.

Species and cultivars	Origin of samples	Biological indexing								ELISA				
		Woody host indexing				Herbaceous host indexing				PPV	PDV	PNRSV	CLRV	ASPV
		PPV	PDV	PNRSV	CLRV	PPV	PDV	PNRSV	CLRV					
<i>Lonicerca caucasicca</i>	Vácraót		X					X					X	
<i>Lonicerca maackii</i>	Vácraót		X					X					X	
<i>Lonicerca sachalinensis</i>	Vácraót		X					X					X	
<i>Prunus cerasifera</i> 'Hollywood'	Budapest			X					X				X	
<i>Prunus cerasifera</i> 'Nigra'	Budapest			X					X				X	
<i>Prunus cerasifera</i> 'Pendula'	Budapest	X						X						
<i>Prunus cerasifera</i> 'Pissardii'	Budapest	X						X						
<i>Prunus glandulosa</i>	Vácraót	X						X						
<i>Prunus glandulosa</i> 'Alba Plena'	Soroksár	X						X						
<i>Prunus glandulosa</i> 'Sinensis'	Vácraót	X						X						
<i>Prunus japonica</i>	Vácraót	X						X						
<i>Prunus mume</i> 'Omori-no-mama'	Vácraót		X										X	
<i>Prunus salicina</i> (from China)	Vácraót		X										X	
<i>Prunus serrulata</i> 'Amanogawa'	Vácraót		X										X	
<i>Prunus serrulata</i> 'Ichiyō'	Vácraót		X										X	
<i>Prunus serrulata</i> 'Pink Perfection'	Vácraót		X					X					X	
<i>Prunus serrulata</i> 'Tshaku'	Vácraót		X					X					X	
<i>Prunus serrulata</i> 'Yedo-sakura'	Vácraót		X		X			X					X	
<i>Prunus sogdiana</i>	Vácraót	X				X					X			X
<i>Prunus spinosa</i> 'Plena'	Vácraót		X					X					X	
<i>Prunus spinosa</i> 'Purpurea'	Vácraót		X		X			X					X	
<i>Prunus subhirtella</i> 'Plena'	Vácraót		X		X			X					X	X
<i>Prunus tenella</i>	Vácraót		X					X					X	
<i>Prunus tomentosa</i> (from Tibet)	Vácraót	X				X				X				
<i>Prunus yedoensis</i>	Vácraót		X		X			X		X			X	
<i>Prunus yedoensis</i> 'Moerheimii'	Vácraót		X		X			X		X			X	
<i>Prunus x bircana</i>	Budapest	X				X				X				
<i>Syringa yunnanensis</i>	Vácraót		X					X					X	

Results and discussion

During 2002 and 2007, 120 ornamental *Prunus* species and cultivars in arboretums/ botanical gardens (288 samples) and in propagation sites (870 samples) were surveyed and sampled. From the samples found to be infected with PPV by ELISA only 7 trees of ornamental *Prunus* species/cultivars showed symptoms affecting the leaf colour. The most conspicuous symptoms of whitish or light yellow rings of differing size, speckles, line patterns or light green vein banding, were observed on the leaves of *P. glandulosa* 'Sinensis' (Fig. 1). On *P. glandulosa* 'Alba Plena' the symptoms were pale and blurred (Fig. 2), and similarly light were the green discoloured areas along the midribs and the sparse blotches on the leaves of *P. japonica* (Fig. 4). Round blotches associated with the veins or long light green discoloured areas were present on *P. cerasifera* 'Pendula' (Fig. 5). *P. cerasifera* 'Pissardii' and *P. cerasifera* 'Woodii' leaves turned

dark-red or purple, mainly along the main and secondary veins, with a higher intensity on the lower surface. The lower leaves of *Prunus x blireana* exhibited similar symptoms along the main, secondary and tertiary veins. On dark-red leaved trees such as *P. cerasifera* 'Nigra' and *Prunus x davidopersica* 'Atropurpurea' symptoms were not visible (Sebestyén et al., 2008).



Fig. 1 PPV-infected *Prunus glandulosa* 'Sinensis' leaves



Fig. 2 PPV-infected *Prunus glandulosa* 'Alba Plena' leaves



Fig. 3 PPV-infected *Prunus glandulosa* leaves



Fig. 4 PPV-infected *Prunus japonica* leaf

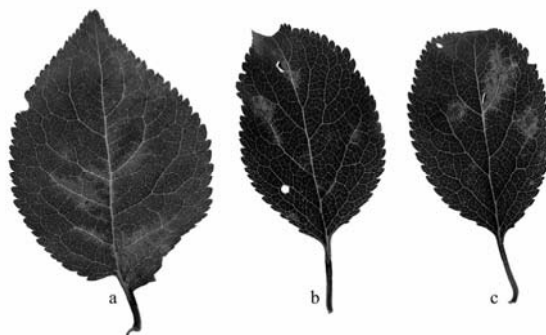


Fig. 5 PPV-infected *Prunus cerasifera* 'Pendula' a) leaf of one-year shoot, b, c) leaves of two-year twig

From 2005 this work has been extended to studying other viruses on other woody plants. Plants (species and varieties) showing virus symptoms among various thousands of woody plants in two botanical gardens and an arboretum were selected and submitted to indexing on woody and herbaceous indicators, as well as to testing with ELISA for the presence of the following 11 viruses occurring on woody plants: PPV, PDV, PNRSV, CLRV, ASPV, ASGV, ApMV, ACLSV, SLRV, TBRV and ArMV. Up to now, among the 28 suspicious species and varieties, PPV, PDV, PNRSV, CLRV and ASPV have been detected so far. In addition to the 9 species/cultivars formerly mentioned as PPV hosts 3 new species/cultivars: *P. glandulosa*, *P. tomentosa* (from Tibet) and *P. sogdiana* were found to be infected by PPV. The symptoms on the leaves of *P. glandulosa* were similar but weaker than those observed on the leaves of *P. glandulosa* 'Sinensis' (Fig. 3). The leaf tissue along the secondary midribs of *P. sogdiana* turned to pale green (Fig. 6). *P. tomentosa* (from Tibet) showed distortion and epinasty on the first leaves; later chlorotic spots developed which became necrotic by mid-summer (Fig. 7).



Fig. 6 PPV-infected *Prunus sogdiana* leaf



Fig. 7 PPV-infected *Prunus tomentosa* (from Tibet) leaves

Seventeen species/varieties showing symptoms were found to be infected with PDV. The symptoms observed were the following: *Lonicera caucasica*: pale green spots and tissue clearing along the veins; *L. maackii*: pale green spots; *L. sachalinensis*: large, transparent, light green spots and tissue-clearing along the veins; *Prunus mume* 'Omoi-no-mama': prominent light green rings and tissue clearing along the veins, leaf distortion (Fig. 8); *P. salicina* (from China): light green ringspots and tissue-clearing (Fig. 9); *P. spinosa* 'Plena': large, transparent, light green spots on the leaves; *P. spinosa* 'Purpurea' no symptoms on the dark red leaves, but heavy gumming appears on the tree trunk; *P. serrulata* 'Amanogawa', *P. serrulata* 'Ichiyo' (Fig. 10); *P. serrulata* 'Pink Perfection' (Fig. 11); *P. serrulata* 'Taihaku' (Fig. 12) and *P. serrulata* 'Yedo-sakura': oak leaf patterns with yellow bands and sparse spots on the leaves; *P. subhirtella* 'Plena': yellow transient spot on the leaves; *P. tenella*: in early springtime transient pale green spots appeared, later and

in most cases it was symptomless - intensive gumming appeared on the twigs of several years old; *P. yedoensis*: prominent, pale green spots and tissue clearing along the midribs, light deformation; *P. yedoensis* 'Moerheimii': conspicuous tissue-clearing mainly along the midribs (Fig. 13); *Syringa yunnanensis*: transient, light green spots on the leaves (Fig. 14).



Fig. 8 PDV-infected *Prunus mume* 'Omoi-no-mama' leaves



Fig. 9 PDV-infected *Prunus salicina* (from China) leaf



Fig. 10 PDV-infected *Prunus serrulata* 'Ichiyo' leaves



Fig. 11 PDV-infected *Prunus serrulata* 'Pink Perfection' leaf



Fig. 12 PDV-infected *Prunus serrulata* 'Taihaku' leaves



Fig. 13 PDV-infected *Prunus yedoensis* 'Moerheimii' leaf



Fig. 14 PDV-infected *Syringa yunnanensis* leaf

Certain species/varieties infected with PDV were positive also for CLRV with ELISA, such as *P. spinosa* 'Purpurea', *P. yedoensis*, *P. yedoensis* 'Moerheimii', *P. subhirtella* 'Plena', *P. serrulata* 'Yedo-sakura' and to ASPV: *P. subhirtella* 'Plena' and *P. serrulata* 'Yedo-sakura'. PNRSV was detected in *P. cerasifera* 'Nigra' and 'Hollywood'. Necrotic flecks and shot-holes were observed on the leaves of infected trees. In the biological indexing the woody indicators and the herbaceous test-plants showed typical symptoms (Németh, 1986) to the viruses detected by ELISA.

The difference of symptoms provoked by PDV on herbaceous hosts suggest that various strains of this virus affect hosts, mainly yellow mottle disease described as a separate strain by Ramaswamy and Posnette on ornamental cherries in 1972.

Conclusions

Based on the six-year study natural PPV infection was found in 12 of the ornamental *Prunus* species/cultivars tested. Only 10 of the 12 naturally PPV-infected *Prunus* species/cultivars/botanical varieties were symptomatic. On infected light red-leaved cultivars symptoms were clearly visible on the lower surface of the leaves, an important consideration for phytosanitary inspectors. On dark red-leaved ornamental *Prunus* cultivars (such as *P. cerasifera* 'Nigra' and *P. x davidopersica* 'Atropurpurea') PPV symptoms could not be recognized even if the virus concentration was high. These latently infected dark red-leaved cultivars can play an important role in the wide distribution of PPV. Such trees in public areas are permanent virus sources. Symptomless infected trees in the vicinity of nuclear stocks and nurseries mean a high risk. Therefore, it is a must for the future to also apply the requirements of the certification scheme for the production of propagating materials to the natural PPV hosts newly identified in this study. Considering the high frequency of latent infections, the use of reliable laboratory methods is essential in regular screening.

During our examinations it was noted that PDV and PPV might cause similar symptoms on the leaves of several *Prunus*, *Lonicera* and *Syringa* species, therefore all the observations should be confirmed by laboratory testing.

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